REVIEW ARTICLE

A Review on Harmful Effects of Phenolic Antioxidants–Zebrafish as a Potential Model to Evaluate Toxicity

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ABSTRACT: The synthetic phenolic antioxidants (SPAs) are widely used as an additives in food, cosmetic and plastic industries to increase the tenability. These antioxidants are degraded into mammals, yet as into the environment like river and water. for instance the common intake of BHT (butyl hydroxytoulene) i.e. one among the kinds of SPAs (synthetic phenolic antioxidants) being is estimated to be 0.3 mg/kg weight, while it absolutely was considered as safe for human at authorized level, but it's ubiquitous presence within the aquatic environment and its controversial toxicological data are of great concern for human similarly as aquatic life. This study summarizes both the synthetic and natural phenolic antioxidants, mainly emphasizing their mode of action, health effects, degradation products and toxicology. Zebrafish (Danio rerio) has prove to be a wonderful vertebrate organism for analyzing various developmental stages, decreasing model, and screening of drug toxicities thanks to their minuscule, high fecundity, optical transparency and their close resemblance with the genome of creature.

KEYWORDS: Synthetic phenolic antioxidants, Zebrafish, Developmental toxicity, Total phenolic content, Drug screening.

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INTRODUCTION

1.1. Water pollution

Water pollution is defined as the release of chemical, inorganic and organic substances into bodies of water that makes water unsafe for human use and disrupts aquatic ecosystems. Water pollution can be caused by a plethora of different contaminants, including toxic waste, petroleum, and disease-causing microorganisms, the release of substances into subsurface groundwater or into lakes, streams, rivers, estuaries, and oceans in such a way that the effluents interfere with beneficial use of the water or with the natural functioning of ecosystems. In addition to the discharge of particles, such as chemicals or microorganisms, water pollution may also include the discharge of energy, in the form of radioactivity or heat, into bodies of water [1].

There are two types of water pollution:

- Organic pollution due to microorganisms such as bacteria and virus present in water, generated by excrement, animal and vegetable waste.
- Chemical pollution which is mostly generated by the nitrates and phosphates of pesticides, human and animal drugs, household products, heavy metals, acids and hydrocarbons used in industries.

1.1.1. What human activities cause the water pollution

Human activities that produce domestic sewage and waste product cause pollution by contaminating water with disease-causing microorganisms and poisonous substances. Oil spills are another source of pollution and causes serious harm to the marine wildlife. Oil spills can effects fishes, get caught within the feathers of birds, mammals and block light from photosynthetic plants within the water.

1.1.2. How does Water pollution effects the aquatic life

Sewage can promote algae growth, which can eventually result in eutrophic "dead zones" where aquatic life cannot survive because of a lack of oxygen. Microplastics are often found in marine wildlife and are mostly consumed by humans as seafood because of biomagnification. Oil spills, such as the Deepwater Horizon oil spill in 2010, which stranded and killed many different marine species.

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1.2 Phenolic Antioxidants

An antioxidant can be defined as a substance that when present at low concentrations, i.e., lower than the oxidizable compound, it significantly delays or inhibits its oxidation. There are 2 basic categories of antioxidants, natural and synthetic, the later ones have been found to cause long-term toxicological effects, including carcinogenicity [2]. Attentions have been paid to both natural and synthetic antioxidants. An Intensive study has been conducted over Antioxidants for the past few decades.

Antioxidants play a vital role against the deteriorating action of free radicals in the organisms. Antioxidants deficiency in living organisms leads to oxidative stress. It has been found that antioxidant compounds present in food, and biological systems are essential in the protection of biomolecules from these free-radical redox reactions [5].

There has been a great interest in scientific communities and consumers regarding phenolic compounds and their antioxidant activity because of the epidemiological studies linking the consumption of diets rich in natural antioxidants with a decreased risk of diseases that are associated with oxidative stress, such as cancer and cardiovascular disease [3]. Phenolic compounds are a large heterogeneous group of secondary plant metabolites that have been widely distributed in plants as bioactive substances and are key constituents of human diet [4]. The natural products which are rich in antioxidants are of great importance for scientists. For this reason antioxidant and antitumor activities of sulfated polysaccharide isolated from marine algae have been studied by many scientists [5].

The most important radical scavengers are the phenolic antioxidants. They convert peroxy radicals into hydroperoxides and are themselves converted to phenoxy radicals. The phenoxy radical can react with another peroxy radical in different ways resulting in nonradical products. However, phenoxy radicals formed are also capable to extract hydrogen from the polymer chain and in this way initiate a new oxidation cycle. The majority of the phenolic antioxidants contain two tertiary butyl groups on the 2nd and 6th positions; these groups are also able to shield the formed phenoxy radical (sterical hindrance) and prevent initiation of a new oxidation cycle. However, introducing sterical hindrance also causes a decrease in radical scavenging rate. Reducing the sterical hindrance may result in an increased radical scavenger activity, but the formed phenoxy radicals may initiate new oxidation cycles and the ratio between the increased radical scavenging rate and the initiation rate will determine the effectiveness of an antioxidant [4].

One of the major changes that usually occur during processing, distribution, and final preparation of food is oxidation. Oxidation of lipids causes other changes in the food system that may affect its nutritional quality, wholesomeness, safety, color, flavor, and texture. Antioxidants are one of the key ingredients that protect food quality by preventing oxidative deterioration of lipids. Auto oxidation of polyunsaturated lipids in food involves a free radical chain reaction that is mostly initiated by exposing lipids to light, heat, ionizing radiation, metal ions, or metalloprotein catalysts. Enzyme lipoxygenase can also initiate oxidation. The classic way of autoxidation includes initiation (production of lipid free radicals), propagation, and termination (production of nonradical products) reactions. Antioxidants can interfere with the process of oxidation by reacting with free radicals, chelating catalytic metals, and also by acting as oxygen scavengers. Phenolic antioxidants mainly function as free radical terminators and sometimes as metal chelators. Phenolic compounds and some of their derivatives are quite effective in preventing autoxidation; however, only a few of such phenolic compounds are currently permissible by law as food antioxidants. The major considerations for acceptability of those antioxidants are their activity and potential toxicity. The approved phenolic antioxidants have been studied extensively for toxicology of their degradation products and yet its still not clear. Some plant phenolic compounds have been considered recently as antioxidants and are being commercially produced such as Flavonoids, the most potent antioxidative compounds of plant phenolics, need further investigation regarding their feasibility of use in foods as well as their toxicological implications. In this review, a thorough discussion of phenolic antioxidants of both synthetic and natural origin, their mode of action, and the active components of naturally occurring plant foods has been done [2].

2. Toxicity

Toxicity is the degree of damage incurred by an organism due to a chemical substance or a particular mixture of substances. Toxicity may be referred as an overall adverse effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell.

Toxicity can be measured generally by its effects on the target (organism, organ, tissue or cell). Because each individual typically have different levels of responses to the administration of same dose of a toxic substance, a population-level measure of toxicity is usually taken into consideration which is related to the probability of an outcome for a given individual in a population. One such measure is the LD_{50}. When such data cease to exist, estimates are usually made through comparison of similar known toxic things, or to similar exposures in similar organisms. Later, "safety factors" are taken into consideration to account uncertainties in data and evaluation processes. For example, consider a dose of a toxic substance to be safe for a laboratory rat, so one might safely assume that one-tenth of that dose would be safe for a human, allowing a safety factor of 10 for inter-species differences between two different mammals; if the respective data is from fish, one might like to use a factor of 100 to account for the major difference between two chordate classes (fish and mammals).Similarly, an additional protection factor can beaded all those individuals who are more likely to be susceptible to toxic effects such as in pregnancy or with certain diseases [6].

Unlike a pure chemical, It is quite more difficult to determine the toxicity of chemical mixtures because each component displays its own toxicity, and further components may interact to produce enhanced or diminished effects. Common chemical mixtures include gasoline, cigarette smoke, and industrial waste. Even more complex will be the situations in which more than one type of toxic entity is involved, such as a discharge from a malfunctioning sewage treatment plant, with both chemical and biological agents. The preclinical toxicity testing on various biological systems reveals the species-, organ- and dose-specific toxic effects of an investigational product. The toxicity of some substances can be observed by studying their accidental exposure to test animals' in vitro studies using cells lines in vivo exposure. Toxicity tests are commonly used to determine specific adverse events or specific endpoints such as cancer, cardiotoxicity, and skin or eye irritation [6].

2.1. Types of systematic Toxic Effects

Toxic effects are categorized basically taking the site of the toxic effect into consideration. In few cases, the effect may take place at only one site. This site is usually termed as the specific target organ.

In other cases, toxic effects may show up at multiple sites. This is known as systemic toxicity. Types of systemic toxicity include:

- Acute Toxicity
- Subchronic Toxicity
- Chronic Toxicity
- Carcinogenicity
- Developmental Toxicity
- Genetic Toxicity

Acute toxicity occurs almost immediately (seconds/minutes/hours/days) after an exposure. An acute exposure is considered to be a single dose or a series of doses received within a period of 24-hours. Death can become a major concern in many cases of acute exposures.

Subchronic toxicity usually results from repeated exposure for several weeks or months. This is a common and we known human exposure pattern for some pharmaceuticals and environmental agents.

Chronic toxicity represents an overall damage to a specific organ system over a period of time which usually takes many months or years to become a recognizable clinical disease. Damage due to individual subclinical exposure may go unnoticed. With repeated exposures or long-term continual exposure, the damage from this type of exposure may slowly builds up (cumulative damage) until the damage exceeds the threshold for chronic toxicity. Ultimately, the damage turns out to be so severe that the organ may no longer function normally [7].

Carcinogenicity is a considered as a complex multistage process involving abnormal cell growth and differentiation that may or fetus. The three basic types of developmental toxicity are:

1. Initiation – a normal cell undergoes irreversible changes.
2. Promotion – initiated cells are stimulated to progress to cancer.

Development toxicity pertains to adverse toxic effects on the developing embryo or fetus. It can results from toxic exposure to either parent before conception or to the mother and her developing embryo or fetus. The three basic types of developmental toxicity are:

1. Embryolethality – failure to conceive, spontaneous abortion, or stillbirth.
2. Embryotoxicity – growth retardation or delayed group growth of specific organ systems.
3. Teratogenicity – irreversible conditions that leave permanent birth defects in life offspring, such as claft palette or missing limbs.

Genetic toxicity results from damage to DNA and altered genetic expression. This process is called as mutagenesis. The genetic change is referred to mutations whereas the agents causing the changes are called a mutagen. There are three types of genetic changes [7].

1. Gene mutation — change in DNA sequence within a gene.
2. Chromosome aberration — changes in the chromosome structure.
3. Aneuploidy or polyploidy — increase or decrease in number of chromosomes.

2.2. Toxicity of Synthetic Phenol Antioxidants

While beneficial health effects have been reported for a few SPAs, such as BHT and BHA, which may have antitumor effects, adverse health effects are more often observed. In this review, we will mainly focus on the adverse health effects of SPAs.

2.2.1 2,6-Di-tert-butyl-4-methylphenol (BHT)

In toxicity studies, BHT is the SPA congener that has received the most attention. An in vitro study with Ishikawa cells demonstrated that BHT can suppress the basal expression of estrogen-responsive genes [8].A 30 day subchronic toxicity study observed dose-related toxic nephrosis in mice fed with 1.35−5.0% BHT in their diets [9]. BHT has been found to enhance the development of tumors induced by dimethylhydrazine [10]. BHT was also found to be an inflammation and tumor promoter in the lungs of male mice dosed with BHT at 150 mg/kg body weight [11]. However, it should be noted that, in a similar study, no carcinogenicity was observed when mice were fed on a diet containing up to 5000 μg /g BHT for 96 weeks [12].

Toxicity effects may also arise from exposure of animals and plants to BHT and its transformation products. Some transformation products have been reported of enhancing toxicity effects with respect to the parent compound. One study had evaluated the acute toxicities of BHT and its transformation products in animals like mice and found LD50 values of BHT (3550 mg/kg) were higher than those of its transformation products (190−2270 mg/kg), suggesting enhanced acute toxicities of the transformation products compared to BHT [13].

2,6-Di-tert-butyl-4-hydroperoxyl-4-methyl-2,5 cyclo-hexadienone (BHT-OOH) has been demonstrated to be a tumor promoter in mouse skin[14].BHT-OOH can be further metabolized to BHT quinone methide[15], which is a very reactive electrophile and can play a significant role in pneumotoxity, hepatoxicity, and skin and lung tumor promotion in mice [16].

A recent in vitro study has shown that a low concentration of BHT-Q (10−6 mol/L) may cause a cleavage of super-coiled DNA through generations of oxygen radicals. BHTCHO can also damage DNA, but it is less toxic compared to BHT-Q, while BHT and several other transformation products such as BHT-OH, BHT-COOH, and BHT-quinol cannot cleave DNA [17]. Finally, in the presence of metal ions, BHT-OOH can cause oxidative damage on DNA directly and BHT-Q can damage DNA indirectly via H_2O_2 generation. In silico methods were also used to determine toxicities of BHT and transformation products. Suggestive results from the ECOSAR program indicated that toxicities of BHT-Q at 3 different trophic levels (green algae, daphnia, and fish) were significantly enhanced compared to BHT [18].

2.2.2. 3-Tert-butyl-4-hydroxyanisole (BHA)

BHA sold commercially usually consists of two isomers: 10% 2-tert-butyl 4-hydroxyanisole (2-BHA) and 90% 3-tert-butyl-4-hydroxyanisole (3-BHA).Most studies that do not differentiate between the isomers appear to refer to 3-BHA[19]. Adverse effects of BHA toxicity have been found in fishes, mice, rats, and human cells. An in vitro study showed that BHA could cause testicular toxicity via mitochondrial dysfunction, endoplasmic reticulum stress, and abnormal calcium levels in the testis [20]. An obvious increase of 17β-estradiol secretion was observed in H295R cells treated with BHA, indicating estrogenic activity by the latter. An in vitro study with calf thymus DNA showed that BHA can interpose in the DNA base pairs. When A549 lung cancer cells were treated with BHA, single-strand DNA breakage and fragmentation in the chromatin and DNA rings were observed, indicating its genotoxicity [21]. Using the 3T3-L1 differentiation model, 3- BHA was found to enhance accumulation of cellular lipid and promote differentiation of adipocytes, whereas 2-BHA had no such effect.BHA can also cause neurotoxicity in human astrocytes by enhancing cytosolic calcium accumulation and endoplasmic reticulum stress [21]. Moreover, BHA caused developmental retardation in zebrafish embryo development in their early stages. In the environment, microplastics could encourage the accumulation of BHA in zebrafish and enhance the developmental toxicity via disturbing the metabolism of arachidonic acid, glycerophospholipid, and lipids [22]. An in vivo study with immature female rats showed that BHA had antiestrogenic activity, leading to a loss of uterine weight in immature female rat. A long-term dietary

exposure (i.e.,18 weeks) study in mice demonstrated BHA to be an obesogen that can affect white adipose tissue accumulation and plasma lipids via transcriptional regulation of adipogenesis, lipid metabolism, and adipocyte endocrine function. Exposure of pregnant mice to BHA in their diets can lead to sleep deficits and subsequent slow learning by their offspring [23]. Chronic dietary intake of BHA (0.5% and 2% of the diet by weight) for two years enhanced the possibility of papilloma and squamous cell carcinoma and hyperplasia in the forestomach of Fisher 344 rats [24].

2.2.3 Propyl 3,4,5-trihydroxybenzoate (PG), Octyl 3,4,5-trihydroxybenzoate (OG), and Dodecyl 3,4,5-trihydroxybenzoate (DG)

An in vitro study showed that PG is cytotoxic in isolated rat hepatocytes, as it can impair mitochondria and lead to ATP depletion [25]. By comparing the cytotoxicities of PG, OG, and DG in rat hepatocytes gives the following order of cyto-toxicities: OG > DG > PG. PG can induce apoptosis in human trophoblast cells through disrupting mitochondrial function; thereby, exposure to PG in early pregnancy may lead to abnormal implantation and placental development. In addition, PG can drastically inhibit human chorionic gonadotropin induced ocyte maturation. In vitro studies with two estrogen-responsive cell lines (MCF-7 and T47D-Kbluc) have demonstrated that PG has high estrogenic activity [26]. In zebrafish, PG demonstrated a developmental toxicity, which induces the formation of ROS and consequent apoptosis. An in vivo study showed that PG can cause testicular toxicity in mice by disrupting mitochondrial or endoplasmic reticulum function and inhibiting testicular development-related genes [27].

2.2.4. Other SPAs

In comparison with the above congeners, much less toxicity data were available for other SPAs. An in vitro study showed that AO246 could lead to lipid disruption by dramatically inducing intracellular accumulation of triacylglycerides in human placental cells, which was related to maternal diabetes and obesity [28]. Long-term exposure to AO246 has been reported to cause liver injury in rats. An in vitro study showed that AO44B25 is a very strong androgen and an estrogen antagonist. DBP, AO2246, and AO425 were also known to have androgen antagonist activity, but not estrogen antagonist activity [29].The susceptibility of newborn rats to DBP has been reported to be 4–5 times higher than that of young rats. An in vivo study further confirmed that prenatal exposure to AO44B25 could majorly affect the central nervous system of male rat offspring; thus, AO44B25 has endocrine disrupting effects during the fetal period. A sub-chronic toxicity study with rats has also reported that AO1076 may induce centrilobular hepatocytic hypertrophy and enhance liver weight, transaminase activity, and cholesterol. In vitro studies showed that TBHQ could intercalate in calf thymus DNA base pairs and cause damage of single strand DNA and fragmentation of chromatin and DNA rings within the nucleus [30]. TBHQ exposure during early stages of zebrafish embryo development may induce organogenesis retardation via interference with pluripotency biomarkers. An in vivo study with male rats showed that the GSH conjugate of TBHQ in the bladder and kidneys could induce carcinogenesis [31].

3. Physicochemical properties, production and uses of Phenolic Antioxidants

Structurally, phenolic compounds comprise an aromaticing, bearing one or more hydroxyl substituent's, and range from simple phenolic molecules to highly polymerised compounds [32]. Despite this structural diversity, the groups of compounds are often referred to as polyphenols. Most naturally occurring phenolic compounds which are present as conjugates with mono- and polysaccharides are linked to one or more of the phenolic groups, and may also occur as functional derivatives such as esters and methyl esters [33]. Though such structural diversity results in the wide range of phenolic compounds that occur in nature. Of these, phenolic acids, flavonoids and tannins are regarded as the main dietaryphenolic compounds [32].

The processing of plant foods results in the production of by-products that are rich sources of bioactive compounds, including phenolic compounds [34]. Phenolic compounds with antioxidant activity have been identified in several agricultural by-products, such as rice hulls [35], buckwheat hulls[36] and almond hulls[37]. Pistachio hulls are yet a source of phenolic antioxidants, and may contain contain up to 34 mg tannic acid equivalents phenolics /g dry weight. Large quantities of peels and seed residue are produced by citrus industry processes, which usually account for up to 50% of the total fruit weight. Citrus industry by-products, if utilized optimally could be a major source of phenolic compounds in the form of peels. As they found it contain higher amounts of total phenolics compared to the edible portions. The total phenolics content in peels of lemons, oranges, and grapefruit were 15% higher than those in the peeled fruits [33].

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertbutylhydroquinone (TBHQ) have been widely used as antioxidants in foods, but concerns over the safety have led towards interests in natural antioxidants [38]. These synthetic antioxidants are substituted phenolic compounds, and subsequently much of the research on natural antioxidants has also focused on phenolic compounds, in particular the flavonoids and hydroxycinnamic acids [39]. The antioxidant activities of phenolic compounds which are extracted from different sources have been studied in several foods and food model systems. Rosemary extracts (carnosol and carnosic acid) were effective antioxidants in corn, soybean, peanut and fish oils tested in bulk systems, but were either ineffective or acted as prooxidants in the corresponding vegetable oil-in-water emulsion systems. This was attributed to the interfacial phenomena, where the hydrophilic rosemary antioxidants remained oriented in the air-oil interphase, thus affording better protection against oxidation. Phenolic compounds extracted from mango seed kernel powder was found to extend the shelf life of buffalo ghee demonstrated that extracts from the hard winter wheat (Triticum aestivum) variety Trego was able to suppress the oxidation of fish oils, and its capacity was comparable to tocopherol [38].

3.2. Physicochemical Properties

SPAs in major share a common structure: phenolic rings substituted by hindered alkyls in the ortho position. The molecular weights of the most frequently used SPAs vary widely, ranging from <200 Da to >1100 Da. The dominant low molecular weight SPA is BHT, whereas the high molecular weight SPAs are represented by pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate) (AO1010) and octadecyl-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate (AO1076) [40]. Representative SPA structures are shown in Figure 1.

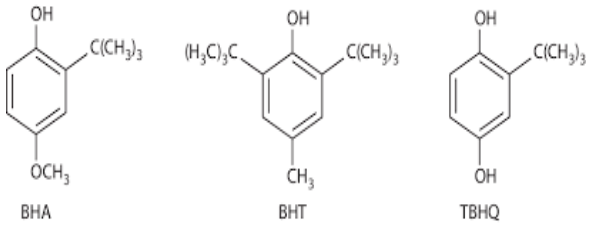

Figure 1: structures of synthetic phenolic antioxidants

Generally, SPAs are hydrophobic chemicals (log K_{ow} = 1.79–17.2) and have low volatility (log K_{oa} = 8.87–39.4). The predicted half-lives of SPAs were in the ranges of 1–75 h, 360–4320 h, 720–8640 h, and 3240–38,900 h in air, water, soil, and sediment, respectively.

3.2. Production and Uses of SPAs

SPAs are synthesized mainly via catalytic alkylation of phenol or cresols with olefins. For example, the industrial synthesis of BHT is mainly via reaction of 4-methylphenol with 2-methylpropene, under the catalysis of sulfuric acid [41]. There is no open data availability on the total global production volume of SPAs. The phenolic antioxidant market globally (for natural and synthetic antioxidants combined) is expected to grow at a rate of 5% annually to reach USD18.3 billion in 2023[42]. The total production volume of SPAs in 1998 in China was reported to be 23 kt [43], but that volume is likely to have increased dramatically in recent years. BHT, the most widely used SPA congener, was synthesized in 1940s to protect petroleum from oxidation. In 2000, the global production capacity of BHT had reached about 62 kt/a[44], with North America being one of the top markets for phenolic antioxidants. The production volumes of high molecular weight SPAs, such as AO1010 and AO1076, are comparable to that of BHT, a low molecular weight SPA; both range from 4536 t to 22680 t. Despite their names, some SPAs have recently been found to have natural sources in addition to industrial ones. For example, BHT can be naturally generated by freshwater phytoplankton (green algae and cyanobacteria) [45]. DBP has been found to be naturally occurring in minimum of 169 species of bacteria, fungi, diatom, liverwort, pteridiphyta, gymnosperms, dicots, monocots, and animals [46], while DTBSBP has been found in the stem of Vernonia amygdalina and AO2246 in the root exudates of sorghum. In addition, DBP was also reported as a degradation product of tris(2,4-di-tert-butylphenyl) phosphite, which is a phosphate antioxidant with high production volume [45].

SPAs can react with free radicals by donating a hydrogen atom in order produce a stable product and thus the corresponding antioxidant free radical. The antioxidant free radical is itself adequately stable and does not appear to initiate or propagate a chain reaction, stopping the autoxidation chain reaction [47]. The applications of the dominant SPA congener, BHT, are better documented. In 2000, 27% of the total BHT produced was used in plastics, 27% in rubber, 17% in fuel additives and mineral oil, 12% in pharmaceuticals, food and cosmetics, and 11% in animal feed, with the remaining 6% being used in printing inks and miscellaneous applications[48]. In recent years, the

low migration tendency of high molecular weight SPAs has led to their preferential use in polymers.

SPAs are used majorly in industrial products, it should also be noted that some SPAs, such as BHT, BHA, and TBHQ, are used as food antioxidants. BHT can be added as single or in combination with BHA or TBHQ to fats and oils (100 mg/kg), chewing gum, seasonings and condiments (200 mg/kg), and other food supplements (400 mg/kg) at different allowable concentrations [49]. However, in recent years due to putative health concerns, some food suppliers such as General Mills have voluntarily stopped adding BHT to food. They also started to use antioxidants such as vitamins A, C, and E, and also extracts of rosemary and thyme to keep certain cereal products fresh [50].

4. Harmful effects of Phenolic Antioxidants - Total environment

SPAs have wide usage in many commercial products especially in the indoor environment. Commercial products that contain SPAs sometimes act as sources for indoor dust. SPAs are not persistent pollutants in the environment. Studies of their environmental behavior have mainly focused on BHT, the dominant SPA congener. The transformation pathways of BHT have been well explored. These investigations have demonstrated that the oxidation of BHT can occur on both the phenolic ring and the alkyl. Oxidation of the phenolic ring can form BHT-Q and BHT-quinol, while oxidation of the methyl group can form BHT-OH, BHT-CHO, and BHT-COOH [51].

4.1. Wastewater Treatment Plants

The behavior of SPAs in waste water treatment plants (WWTPs) has been investigated in several countries. Investigations on the behavior of SPAs in a WWTP activated sludge system consisting of anaerobic, anoxic, and oxic tanks (AAO) in China. Six SPAs (BHT, AO246, DTBSBP, BHA, 4-tOP, andAO2246) were detected in the WWTP influent, with high removal efficiencies (80.1−89.2%) in the AAO treatment system, mainly by sludge absorption. Notably, three BHT transformation products were observed to have removal efficiencies far below zero (−365% to −178%) in the AAO system, indicating the formation of these transformation products from BHT. Lu et al. investigated the outcome of 13 SPAs, including BHT, DBP, and AO246, in different types of WWTPs (aerated lagoon, chemically assisted primary treatment, secondary biological treatment, and advanced biological nutrient removal treatment) in Canada[52].The removal efficiencies of total SPAs were in the range of 43.4–99.9%(median: 99.1%), with no significant differences found among the four types of WWTPs. Wang and Kannan reported the occurrence and fate of BHT and four metabolites (BHT-OH, BHT-CHO, BHT-COOH, and BHT-Q) in two WWTPs in Albany, New York State, both of which used an activated sludge system consisting of an aeration tank [53]. The removal efficiencies of BHT in the two WWTPs ranged from 62.3% to 76.2%. Similarly to the study in China by Liu et al., the removal efficiencies of BHT transformation products in WWTPs in Albany were also reported to be far below zero (−3020% to −65.6%). Based on the allowed concentration of BHT in personal care products (0.1% by weight, that is, 1000 μg/g), Wang and Kannan estimated that personal care product use could contribute 91% of the total mass loading of BHT in WWTPs in New York. Based on the mmc of BHT in 214 personal care products sampled in Toronto (0.25 μg/g), Liu and Mabury estimated that personal care products accounted for 10% of the BHT loading to WWTPs in Toronto. Witthayaphirom et al. investigated the removal of BHT by constructed wetlands (cattail vegetation) with different reactive media (sand, clay, and iron powder).Adsorption and biodegradation were the primary mechanisms of removal, while plant accumulation was relatively trivial. Adsorption and biodegradation efficiencies for BHT (73.8%) were at their maximum when sand, clay, and iron powder were used in combination [54].

In addition to the above primary and secondary treatment systems, the use of advanced oxidation processes to degrade BHT has also been investigated. Rodil et al. [55] found that via chlorination, BHT can be oxidized to BHT-Q, which is relatively quite stable. In comparison with BHT, BHA is more easily oxidized by chlorination, which results in a shorter half-life for BHA. The oxidation of BHT by potassium permanganate has also been investigated, and its found to lead in the formation of BHT-Q and several other unusual products, such as BHT-dimer. The oxidation of BHT by bromine can produce BHT-CHO, 2,6-di-tert-butyl-4-methyl-4-methoxy-2,5- cyclohexadienone, and 3,5-di-tert-butyl-4-hydroxybenzyl acetate, with 2,6-di-tert-butyl-4-bromo-4-methyl-2,5-cyclohexadienone as an intermediate. The ozone microbubble technique was demonstrated to oxidize BHT more efficiently than the conventional ozone technique due to improved ozone gas transfer [56]. Various compounds including BHT-quinol and BHT-Q were identified as possible transformation products in the ozone oxidation study. Advanced oxidation of AO2246 in landfill leachate by a composite nano-Fe3O4 material catalyzing micro-ozonation system has also been investigated [57]. Under optimal conditions, removal of more than 70% of AO2246 was achieved in 120 min. Transformation products of A02246 identified in the study included 3,5-bis(1,1-dimethylethyl)- phenol, 4-(1,5-dihydroxy-

2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one, and other small polar compounds.

4.2. Degradation in Soil and Aquatic Environment

The biodegradation of BHT in soil has been investigated using C mass labeled standard.BHT was found to be labile in soil: 84–89% of the dosed BHT was biodegraded in 24 h, and >95% of the dosed BHT was biodegraded after 24 days [58]. Two major metabolites, 4-hydroperoxy-4-methyl-2,6-di-tert-butyl cyclohexa-2,5-dienone (BHT-OOH) and BHT-quinol, were observed, while products resulting from oxidation at the methyl group (BHT-OH, BHT-CHO, and BHT-COOH) were identified as minor metabolites[59]. Similar BHT biodegradation pathways have been observed in water and activated sludge, with BHT-OOH, BHT-quinol, BHT-OH, BHT-CHO, BHTCOOH, and unknown polar chemicals identified as metabolites. In silico tools can be useful to detect outcome of chemical transformation mechanism. Using density functional theory, Wang et al. tested theoretical calculations to guess the outcome of transformation mechanism of BHT initiated by OH radicals in aquatic environment. OH-addition reactions on phenolic ring of BHT were found more likely to occur in comparison to abstraction reactions. The in-silico predictions may help to identify new transformation products in the environment [60].

5. Role of Zebrafish in Toxicity research

Initially, within the early 1970s, the interest on zebrafish was as a model system, when it absolutely was selected to develop the primary vertebrate assay enabling forward screening [61]. During the next 30 years, zebrafish was almost accustomed study organ development. This resulted within the categorization of enormous number of genes involved in vertebrate pathways, which helpedin the identification of zebrafish as most reliable model in human disease and pharmaceutical researches. For pharmacology investigations the lucrative feature of zebrafish assays is its prospect in using them as medium-to-high throughput screening mode, for its small size (5 cm for an adult and 5 mm for 7 days post-fertilization (dpf) larvae) and robust fish that's easy to keep up because of their high fecundity [63].

Earlier zebrafish are used for evaluating the toxicity of agrochemical agents[62] but more recently, their use for toxicity assessment of pharmaceutical compounds has been greatly increased[63]. An in vivo toxicology evaluation of zebrafish larvae are reached in an exceedingly week; since comparable mammalian assays requires shorter time frames. Toxicology studies often reveal effects that require further investigation in explaining purposes that are costly and time taking. Screening technologies exist and are being further developed in zebrafish, which should provide very early details of potential off-target effects on the cardiac system additionally as other functions like effects on brain system, intestinal tract, olfactory and other sensory functions, pro-convulsant potential and bone formation. Hence, the zebrafish technology has to be taken into consideration as a effective pre-filter to support selection of the simplest safe candidates for fast yet feasible ways within the drug discovery process.

The zebrafish turns out to be a highly acceptable model in toxicity studies of antipsychotics most suited for these reasons. Although mammalian toxicity studies are still considered the gold standard for risk assessment, the zebrafish is still most considered as valid model forits toxic responses that appear to be well conserved between mammalians and zebrafish. The life cycle of the Zebrafish and its development is shown in figure 2.

Understanding syndromes with genetic bases has become a very important topic in medicine, with the hope that new treatments are discovered. The zebrafish appears as a quick model to review de novo mutations and genetic diseases. Using genomic editing approaches as CRISPR/Cas9, or artificial site-specific nucleases like zinc-finger nucleases, and transcription activator-like nucleases, genes may be inactivate in vivo, mimicking human phenotypes, and obtaining information about human diseases with genetic background.

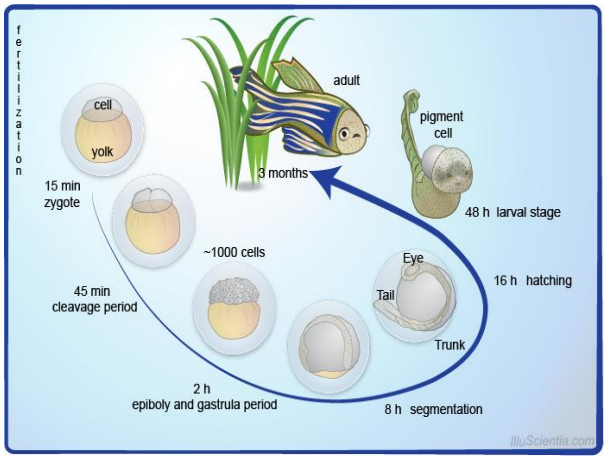

Fig 2: Zebrafish- characteristics, life cycle and development.

5.1. Advantages of zebrafish model taking cost and time into consideration

As it has been described, toxicity testing of drugs in recent years has employed various animal models [64]. Although mice, rats, rabbits and dogs are excellent models according to most standards, they present some serious limitations. Usually experiments conducted in evaluation of drug toxicity involves requirement of animals in large numbers which result in increase of monetary cost of the experiments significantly and also handling of these animals is quite time consuming. Whereas, the small size of zebrafish make them, ideal for experiments, as they are easy to handle and low costs providing researchers and those concerned with animal welfare, with an alternative to work according to the 3Rs principles (refinement, reduction and replacement)[65]. In comparison to mammals of similar kind, zebrafish has the more potential to disclose the pathways of developmental toxicity. Zebrafish therefore, provides a sound basis for the risk assessment of drug administration in humans. Thus, in many respects, the use of the zebrafish as a model for studies of cardio- behavior, genotoxicity or hepatotoxicity would allow the researcher to overcome many of the challenges presented by using other animals models, including limitations on sample size and higher monetary and time costs [66].

5.2. Evaluation of toxicity by Zebrafish

Toxicology studies helps in determination of the suitability and consequences of drug administration in humans. one of the foremost concerns of drug discovery process is evaluation of drug hepatoxicity,its assessed using preclinical cell culture, animal models and clinical trials. However, drug hepatotoxicity is difficult to detect before human use, limiting the invention and development of novel therapies using conventional models. As a result of this fact, to develop new in vivo and in vitro models for efficacy and safety testing is required. It helps in founded of upper tools in screening of drug-induced liver injury (DILI) of enormous compound libraries within the first stages of drug development that helps in better understanding of hepatotoxicity. Being this fact the foremost common explanation for withdrawal Zebrafish liver organogenesis starts at 3 days post fertilization (dpf) and is fully functional by 5 dpf. The tri-lobed liver of the zebrafish's biological function is most almost like other mammal, including the processing of vitamins, lipids, carbohydrates and proteins, also because the serum protein synthesis. Some studies even suggest that metabolism of medication during exposure to zebrafish embryos is analogous thereto in humans [67].

5.3. Drug screening using Zebrafish

Cardiovascular toxicity could be a major limiting consider drug development and requires multiple cost-effective models to perform toxicological evaluation [68]. Zebrafish is now became a well-validated model in the study of treatment with minute molecules, yet on elucidate biological functions, and in deciphering the mechanism of bioactive compounds. The model has emerged as a robust system for little molecule screening and for novel biological and therapeutic discoveries [69]. As an example through in place hybridization is also reported the expression of some target genes [70]. That assay required prior knowledge of the biologic process and also relies on the chosen molecular target, which is critical for the developmental events.

In a shot to guage toxicity of medicaments and other chemicals, new methodologies that concentrate on cardiomyocyte properties or computational models are under development. Despite their usefulness, these strategies fails to produce sufficient information regarding toxicity within the organism as a results of the secondary metabolism that's derived from the drugs, or the drug effects in other cellular lines present within the heart[71]. Primary cardiomyocytes derived from human embryonic stem cells are accustomed evaluate cardiotoxicity; however the consensus is that a reliable in vivo model is required. Additionally some in silico approaches (computational techniques) can assess the ionic flow by the action of a drug, reaching some results about Na+, K+, L-type Ca2+ channels or multiple membrane ion channels in cardiomyocytes. These computational models appear as cardio toxicological method in evaluating the actions of medication on cardiac electrical activities at cellular and tissue [72].

Cardiac electrophysiology and modeling drug-channel studies, have documented many improvements in the last decades, although, the generation of a virtual heart model for drug safety assessment is still a major challenge. Firstly, more studies are necessary to develop biophysically accurate models: the zebrafish could constitute a good approach for drug-channel interactions and cardiac electrophysiology [72]. Zebrafish are complex vertebrates but simpler in comparison to humans that maintain equally elaborate mechanisms in activating or relieving the effects of exogenous chemicals. As it is close resemblance of the genetic cascade that governs the heart development in zebrafish similar to humans has proven the zebrafish system as a cost-effective model to conduct pharmacological screens on developing embryos and larvae as well as to provide data to generate computational models to evaluate *insilico* drugs studies [73].

6. CONCLUSION

Toxicity effects of some SPAs like BHT and BHA are studied and there's limited or no information on the toxicity effects of the many SPAs. Several phenolic antioxidants and other contaminants are detected simultaneously within the environment and in humans. Finally the long run studies should be to develop novel phenolic antioxidants with low migration abilities Zebrafish may be accustomed assess the toxicity of drug candidates in early screening assays, sometimes in a very high-throughput manner. Because of their small size and their transparency, such testing requires a tiny amount of mass test article, little or no laboratory space, and data will be collected from noninvasively over time in vivo. Although maintenance of zebrafish is relatively easy, yet it a complex process, greatly influenced by various factors including mating behavior, day-light cycle, age, and size of the fish. These data can help to prioritize safe compounds for mammalian testing, disclose mechanism of toxicity, and identifies cotherapies that mitigate toxicity of promising therapeutics.

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